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Fractionation of Bagasse into Cellulose, Hemicelluloses, and Lignin with Ionic Liquid Treatment Followed by Alkaline Extraction

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ABSTRACT: Lignocellulose materials are potentially valuable resources for transformation into biofuels and bioproducts. However, their complicated structures make it difficult to fractionate them into cellulose, hemicelluloses, and lignin, which limits their utilization and economical conversion into value-added products. This study proposes a novel and feasible fractionation method based on complete dissolution of bagasse in 1-butyl-3-methylimidazolium chloride ([C_4mim]Cl) followed by precipitation in acetone/water (9:1, v/v) and extraction with 3% NaOH solution. The ionic liquid [C_4mim]Cl was easily recycled after concentration and treatment with acetonitrile. ^{1}H NMR analysis confirmed that there was no obvious difference between the recycled [C_4mim]Cl and fresh material. Bagasse was fractionated with this method to 36.78% cellulose, 26.04% hemicelluloses, and 10.51% lignin, accounting for 47.17 and 33.85% of the original polysaccharides and 54.62% of the original lignin, respectively. The physicochemical properties of the isolated fractions were characterized by chemical analysis, high-performance anion exchange chromatography (HPAEC), gel permeation chromatography (GPC), Fourier transform infrared (FT-IR), and ^{1}H and 2D ^{13}C−^{1}H correlation (HSQC) nuclear magnetic resonance spectroscopy. The results showed that the acetone-soluble lignin and alkaline lignin fractions had structures similar to those of milled wood lignin (MWL). The easy extraction of the noncellulose components from homogeneous bagasse solution and amorphous regenerated materials resulted in the relatively high purity of cellulosic fraction (>92%). The hemicellulosic fraction was mainly 4-*O*-methyl-D-glucuronoxylans with some α-L-arabinofuranosyl units substituted at C-2 and C-3.

KEYWORDS: bagasse, ionic liquid, dissolution, fractionation, cellulose, lignin, hemicelluloses

INTRODUCTION

Lignocellulosic biomass, such as agricultural residues, forestry wastes, waste paper, and energy crops, represents a potentially sustainable source to create fuels, chemicals, composites, and a host of other products to replace fossil-based products.[1,2] The main chemical components in lignocellulosic materials are cellulose, hemicelluloses, and lignin. Cellulose, a linear homopolymer of β-1,4-glucopyranose units with numerous inter- and intramolecular hydrogen bonds, is highly resistant to dissolution in water and common organic solvents. Hemicelluloses, however, are branched short-chain polysaccharides with an amorphous structure comprising different monosaccharides, which vary among plant species. Lignin is the major nonpolysaccharide heteropolymer consisting of phenylpropanoid units with various degrees of oxygenation/substitution on the aromatic ring. It forms a three-dimensional network and is also covalently linked to hemicelluloses.[3] The high crystallinity of cellulose, complex chemical cross-linking between components, and sheathing of cellulose by hemicelluloses and lignin all contribute to the recalcitrance of lignocellulose to chemical treatment, which limits its utilization and prevents economically feasible conversion into value-added products.[4] The current typical lignocellulose application pattern is based on the product streams that (I) all of the separated saccharides and cellulose are used to produce cellulosic bioethanol or other biobased products and (II) all of the residues, including lignin and residual cellulose and hemicelluloses, are discarded or burned to generate power.[5] In this biomass refinery pattern, the feedstock is only partially available for use. It causes environmental pollution and resource underutilization. To overcome the drawbacks of the conventional biomass refinery pattern, more economically feasible and environmentally friendly integrated utilization and multiproduct biorefinery of lignocellulosic biomass are suggested. In this pattern, effective fractionation pathways are significant prerequisites and play a key role in lignocellulose utilization.

Ionic liquids (ILs), also known as room temperature ionic liquids (RTILs), are organic salts consisting entirely of ions. They possess many advantages such as high thermal stability, negligible vapor pressure, low flammability, and recyclability.[6] Moreover, the physical and chemical properties of these "green" solvents can be tailored by changing the cation and/or anion to meet the required application. Previous studies showed that cellulose could be dissolved in many kinds of ILs with different structures, such as 1-butyl-3-methyl- and 1-allyl-3-methylimidazaolium chloride ([C_4mim]Cl and [Amim]Cl),[7,8] 1-ethyl-3-methylimidazolium acetate ([C_2mim]Ac),[9] 1,3-dialkylimidazolium formates,[10] and 1-ethyl-3-methylimidazolium phosphate.[11] It is believed that the ability of ILs to break the extensive hydrogen bond network in cellulose is crucial to the dissolution process. The presence of water (>1 wt %) significantly decreases the solubility of cellulose in ILs by competitive hydrogen bonding to

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cellulose microfibrils.[7] In addition, many other biopolymers such as hemicelluloses,[12] lignin,[13] chitosan,[14] starch,[15] protein,[16] and β-cyclodextrin[17] can also be dissolved in ILs. Very recently, interest in application of ILs in biomass has been centered on processing of lignocellulosic materials, including dissolution and subsequent modification, characterization, and fractionation. Up to 8% of Norway spruce sawdust could be dissolved in [C_4mim]Cl and [Amim]Cl at 110 °C within 8 h and was easily regenerated as amorphous materials by precipitation in water or organic solvents such as ethanol, acetone, and acetonitrile.[18] Selective extraction of lignin from lignocellulosic materials with ILs has also been achieved. [C_2mim]$^+$-based ILs containing a mixture of alkylbenzenesulfonates with xylenesulfonate as the main anions was used to extract lignin from sugar cane bagasse at 190 °C and atmospheric pressure.[19] The yield of lignin was over 93% (based on the original lignin content), and that of cellulose pulp was over 46% (based on the original bagasse weight). However, most of the hemicelluloses were hydrolyzed into mono- and oligosaccharides. Furthermore, both softwood and hardwood in small particles (0.125−0.250 mm) could be completely dissolved in [C_2mim]Ac after long treatment. Then carbohydrates and lignin could be separated in acetone/water (1:1, v/v). Partial carbohydrate-free lignin (38% based on the original lignin) was successfully recovered by evaporation of acetone.[20] In this dissolution and fractionation process, however, substantial amounts of carbohydrates and lignin (42% of the original wood) were lost.

So far, no method has been reported to fractionate lignocellulosic biomass into cellulose, hemicelluloses, and lignin using IL as a medium. In this study, we present the fractionation of cellulose, hemicelluloses, and lignin from ball-milled sugar cane bagasse after complete dissolution in [C_4mim]Cl. The physicochemical properties of the fractions obtained were characterized with chemical analysis, high-performance anion exchange chromatography (HPAEC), gel permeation chromatography (GPC), Fourier transform infrared (FT-IR), and ^{1}H and 2D ^{13}C−^{1}H correlation (HSQC) nuclear magnetic resonance spectroscopy.

MATERIALS AND METHODS

Materials. Sugar cane bagasse was kindly provided by a local factory (Guangzhou China). It was dried in sunlight and then cut into small pieces. The cut bagasse was ground and screened to prepare 40−60 mesh size particles and dried in a cabinet oven with air circulation for 16 h at 55 °C. The dried bagasse was extracted with toluene/ethanol (2:1, v/v) and further pulverized with a vibratory ball mill for 72 h in a stainless steel jar.

[C_4mim]Cl with 99% purity was purchased from Cheng Jie Chemical Co., Ltd., Shanghai, China. All other solvents were of analytical reagent grade and directly used without further purification. Microcrystalline cellulose (MCC) with a particle size of 50 μm was obtained from Daojun Trading Co., Guangzhou, China. Xylan from oat spelts with over 80% purity was provided by Sigma-Aldrich. Milled wood lignin (MWL) from bagasse was obtained by extraction of ground powder with 96% dioxane three times followed by concentration and precipitation of the resulting solution.

Bagasse Dissolution and Fractionation. To isolate cellulose, hemicelluloses, and lignin from bagasse, a fractionation pathway was performed according to the scheme in Figure 1. Half a gram of ball-milled bagasse was added to 25 g of [C_4mim]Cl at 110 °C under a nitrogen atmosphere with agitation to obtain a clear solution. The resulting bagasse/[C_4mim]Cl solution was poured into 200 mL of acetone/water (9:1, v/v) under vigorous agitation. After centrifugation at 4000 rpm for 15 min, the solid residue (residue 1) was collected and washed thoroughly with acetone/water (9:1, v/v) to eliminate [C_4mim]Cl. The supernatant was collected and concentrated and then added into 250 mL of acidified water (pH 2.0) with stirring. The solid residue (residue 2) was centrifuged and washed with acidified water (pH 2.0). Dried residue 1 was extracted with 3% NaOH aqueous solution for 45 min at 50 °C with a solid to liquid ratio of 1:25 (g mL^{-1}). The insoluble residue (residue 3) was collected by centrifugation and washed with distilled water three times. The filtrates were mixed and adjusted to pH 6.8 with 4 M HCl and then precipitated in 3 volumes of 95% ethanol. The resulting residue (residue 4) was filtered out followed by repeated rinsing with 95% ethanol. The filtrate and wash were mixed and concentrated under reduced pressure to remove ethanol. The concentrated solution was introduced into 50 mL of acidified water (pH 2.0). The resulted residue (residue 5) was filtered out followed by thorough rinsing with acidified water (pH 2.0). All of the residues obtained (residues 1−5) were freeze-dried for analysis.

Reuse of IL. Filtrate 2, containing water, IL, hydrochloric acid, and some degraded carbohydrates and lignin, was adjusted to pH 9.0 with 1 M NaOH. Most of the water was removed by evaporation under reduced pressure. Then acetonitrile was added to the resulting mixture to dissolve IL and leave sodium chloride as an insoluble residue. After filtration, the resulting liquor was evaporated under reduced pressure to remove acetonitrile and then dried at 60 °C under vacuum for 24 h to obtain the recycled IL.

Chemical Characterization. The composition of neutral sugars and uronic acids in the original bagasse and the polysaccharide fraction (residues 3 and 4) was determined by HPAEC. Samples were hydrolyzed with 10% H_2SO_4 for 2.5 h at 105 °C and then diluted 50-fold, filtered, and injected into the HPAEC system (Dionex ISC 3000) with an amperometric detector, an AS50 autosampler, a CarbopacTM PA-20 column (4 × 250 mm, Dionex), and a guard PA-20 column (3 × 30 mm, Dionex). Neutral sugars and uronic acids were separated in a 5 mM NaOH isocratic mixture (carbonate free and purged with nitrogen) for 20 min, followed by a 0.75 mM NaOAc gradient in 5 mM NaOH for 15 min. Total analysis time was 50 min, and the flow rate was 0.4 mL/min.

The acid-soluble and acid-insoluble lignin contents of original materials and residues 1, 3, and 4 were measured according to NREL methods.[21] Molecular weights of MWL and the lignin fractions (residues 2 and 5) after acetylation were determined by GPC on a PLgel 5 μ 500 Å column (Agilent Technologies, U.K.) with a Wyatt Dawn Heleos-II detector. Approximately 50 mg of lignin was acetylated in an acetic anhydride/pyridine solution (1:1, v/v) overnight. Then 3 mg of acetylated sample was dissolved in 9 mL of tetrahydrofuran, and 100 μL of the resulting solution was injected. The column was operated at 40 °C and eluted with tetrahydrofuran at a flow rate of 1 mL/min.

Spectroscopic Characterization. FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet 510) using a KBr disk containing approximately 1% finely ground samples. Thirty-two scans were taken for each sample with a resolution of 2 cm^{-1} in transmission mode in the range of 4000−400 cm^{-1}.

^{1}H NMR and ^{1}H−^{13}C correlation 2D NMR (HSQC) spectra were recorded on a Bruker AV-III 400 MHz spectrometer. ^{1}H NMR spectra of the fresh and recycled [C_4mim]Cl were recorded in $CDCl_3$. ^{1}H NMR spectra of hemicelluloses were acquired using 20 mg samples in 1.0 mL of D_2O. HSQC spectra were taken at room temperature with 50 mg of acetylated sample in 0.5 mL of $CDCl_3$. ^{31}P NMR spectra of the fresh and recycled IL were recorded a Bruker DRX 400 NMR instrument based on the previously reported method.[22,23] Half a gram of IL and 150 μL of pyridine were placed in a 5 mL vial, and the mixture was shaken to form a transparent solution. Then 200 μL of 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane was added and shaken until a yellow paste was formed. After the addition of $Cr(acac)_3$/$CDCl_3$ solution (1 mg/mL, 500 μL), the sample was shaken until it was homogeneous. Then, 200 μL

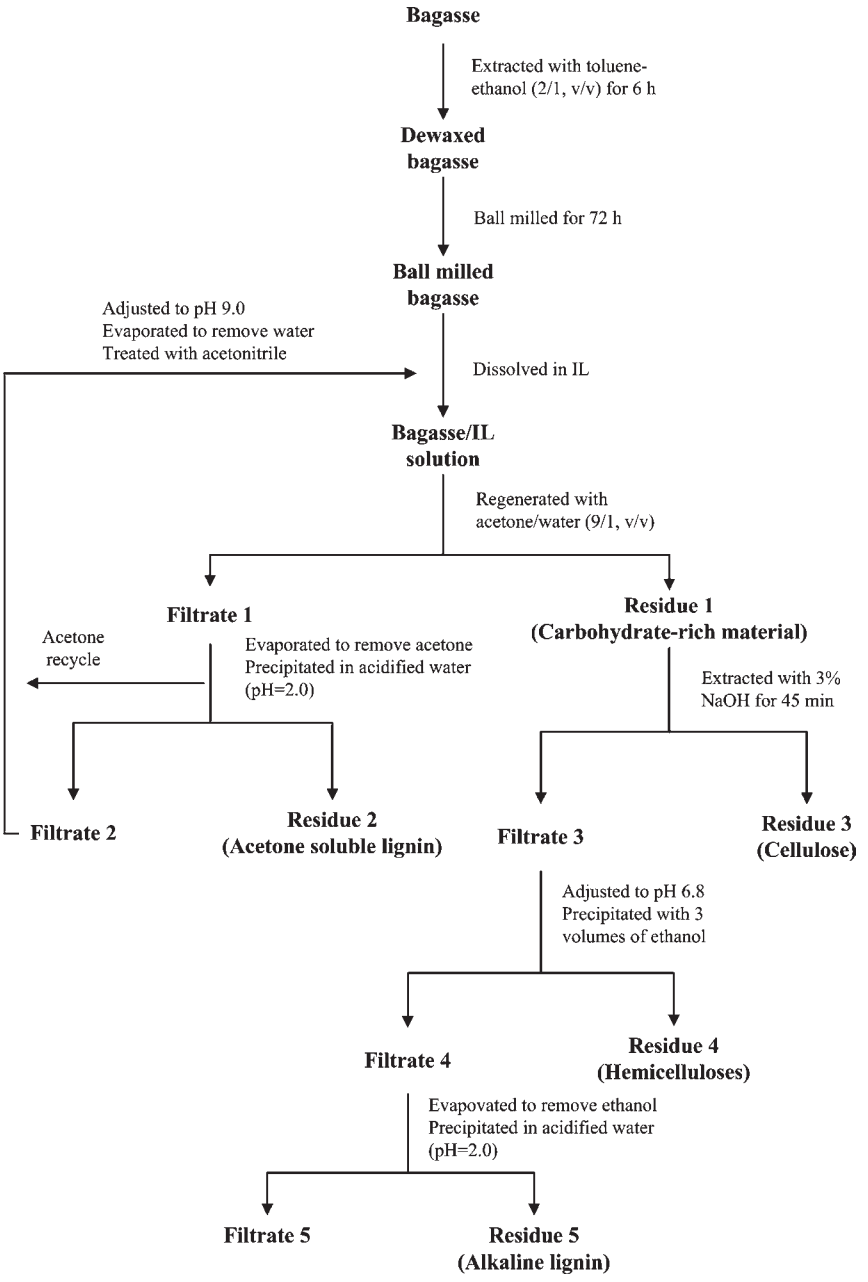

Figure 1. Schematic process of bagasse fractionation based on complete dissolution in $[C_4mim]Cl$ followed by precipitation in acetone/water (9:1, v/v) and extraction with 3% NaOH.

of internal standard *N*-hydroxy-1,8-naphthalimide solution (63.2 mM, 13.47 mg/mL in 3:2 pyridine/$CDCl_3$) was added. The resulting solution was further diluted by $Cr(acac)_3/CDCl_3$ solution (1 mg/mL, 500 μL). The ^{31}P NMR spectra were immediately recorded with 800 μL samples.

The wide-angle X-ray diffraction pattern of the native bagasse, ball-milled bagasse, and isolated cellulose (residue 3) was recorded on a Bruker (German) D8 ADVANCE X-ray diffractometer equipped with Ni-filtered Cu Kα_1 radiation (λ = 0.154 nm) at room temperature. The scattering angle range was 5−40° with 8°/min scanning speed and a 2θ step interval of 0.02°.

RESULTS AND DISCUSSION

Dissolution and Fractionation in IL. The contents of neutral sugars, uronic acids, lignin, and ash in the extractive-free bagasse were determined according to the standard NREL methods. The results showed that bagasse contained 77.98% polysaccharides (including 44.85% glucose), 19.24% lignin, and 0.51% ash. Cellulose accounted for about 57.51% of polysaccharides, estimated from glucose, and the other 42.49% was hemicellulosic polysaccharides. Xylose (29.75%) was the predominant sugar component in bagasse hemicelluloses. Uronic acid (1.49%), arabinose (1.44%), and galactose (0.45%) were observed as minor constituents. Rhamnose and mannose were detected at only trace amounts. These data indicated that xylan with uronic acid and arabinose attached to the main chain was the major hemicellulose in bagasse.

It was reported that $[C_4mim]Cl$ had high ability to dissolve lignocelluloses. It could partially dissolve wood shavings,[24] and

even completely dissolve sawdust (up to 8%) at 110 °C within 8 h.[18] In the present study, [C_4mim]Cl was used as solvent to dissolve the 72 h ball-milled bagasse at 110 °C. Complete dissolution was achieved within 4 h. The decrease in dissolution time was probably due to the low density and loose structure of bagasse compared with wood materials, resulting in the improved accessibility of bagasse and increased penetration and diffusion of IL into its interior. The similar increasing trends of dissolution efficiency of three wood species following their density was also reported as the order of *Eucalyptus grandis* < Norway spruce thermomechanical pulp (TMP) < southern pine.[22] Many more difficulties in the dissolution of hardwoods in [C_4mim]Cl than softwoods were also reported to be due to the tighter structure of hardwoods.[23]

On the basis of the complete dissolution of bagasse in [C_4mim]Cl and the significantly different solubilities of cellulose, hemicelluloses, and lignin in acetone and alkaline solutions, the fractionation process, as shown in Figure 1, was designed. To reduce errors and confirm the results, each experiment was performed three times under the same conditions, and the yields represent the average value. After dissolution of bagasse in [C_4mim]Cl, the resulting solution was introduced into acetone/water (9:1, v/v) to partially dissolve lignin and regenerate the carbohydrate-rich fraction (residue 1). This part of lignin (residue 2) could be recovered by evaporation of acetone and precipitation in acidified water (pH 2.0). The yields of residues 1 and 2 were 84.34 and 6.54%, respectively. The other part of materials accounting for 9.12% was degraded or lost during the dissolution and filtration process and was not collected as products. Because the restrictions of cell wall structure, high cellulose crystallinity, and cellulose sheathing with the hemicelluloses−lignin network were totally destroyed after dissolution in [C_4mim]Cl, the formed bagasse/[C_4mim]Cl solution was homogeneous, which made it possible to easily extract lignin without linking to carbohydrates using lignin solvent such as acetone/water (9:1, v/v). However, the lignin linked to carbohydrates with chemical bonds was hardly isolated with this method. It is generally accepted that alkali treatment could effectively cleave the α-ether linkages and the ester bonds between lignin and hemicelluloses, resulting in the dissolution of hemicelluloses and lignin.[25] In present study, therefore, residue 1 was extracted with 3% NaOH to dissolve hemicelluloses and lignin, and the resulting residue (residue 3) was cellulose. The dissolved hemicelluloses were precipitated with 3 volumes of 95% ethanol at pH 6.8 and recovered by filtration (residue 4). Alkali-soluble lignin (residue 5) was obtained after removal of ethanol followed by precipitation in acidified water (pH 2.0). This extraction released 3.97% lignin and 26.04% hemicelluloses, corresponding to 20.63% of the original lignin and 33.85% of the original polysaccharides, and yielded 36.78% cellulose as residue, corresponding to 47.17% of the original polysaccharides and 82.00% of the original cellulose. About 17.55% of the original bagasse was lost during alkaline extraction and the following filtration and washing operation, which was not recovered as products. Taken together, bagasse was fractionated to 36.78% cellulose, 26.04% hemicelluloses, and 10.51% lignin, accounting for 47.17 and 33.85% of the original polysaccharides and 54.62% of the original lignin, respectively, and 26.67% of the original bagasse was lost; that is, over 73% of the original bagasse was recovered. Similar results were reported in the extraction of lignin from wood in IL,[20] and 42% of the original wood was lost. Under the same fractionation conditions, eucalyptus was fractionated to 38.66% cellulose, 19.71% hemicelluloses, and 6.41% lignin, accounting for 57.69 and 29.41% of the original polysaccharides and 21.06% of the original lignin, respectively (not shown in this paper, but will be published elsewhere). These results indicated that the relatively loose structure and low density of bagasse resulted in the higher accessibility of components and more successful fractionation than with eucalyptus. This result was in agreement with the decreased pretreatment efficiency of lignocelluloses using ionic liquid [Amim]Cl with increased wood density.[23] In addition, both [C_4mim]Cl and acetone could be easily recycled in this fractionation process due to their huge difference in volatility.

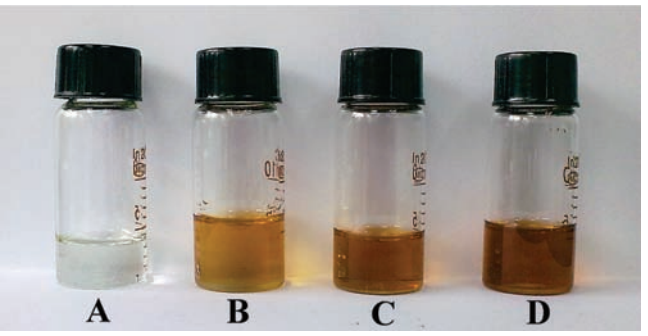

Figure 2. Change of [C_4mim]Cl color with increased recycles (A, fresh; B, first cycle; C, second cycle; D, third cycle).

Table 1. Regeneration of Bagasse from the [C_4mim]Cl Solution in Acetone/Water (9:1, v/v) with Increased [C_4mim]Cl Recycle Number

recycle no.	yield of regenerated bagasse (residue 1, %)	total lignin content in the regenerated bagasse (%)
0	84.34	10.64
1	81.23	12.56
2	78.96	13.06
3	79.80	12.67

Reuse of [C_4mim]Cl. The recovery and reuse of ILs is one of the main challenges for the industrial utilization in view of environmental concerns. In the present study, [C_4mim]Cl was easily recycled by washing the concentrated IL solution with acetonitrile followed by simply evaporating and drying. The yield of the recycled [C_4mim]Cl was over 95%. In addition, the colorless [C_4mim]Cl became amber after recycling. The color was probably due to the contaminants present in [C_4mim]Cl, which could be degraded from [C_4mim]Cl and lignocelluloses or even introduced with chemicals, water, and other solvents. Figure 2 shows the change of [C_4mim]Cl color with increased recycles. Clearly, the color was increasingly dark with increased reuse. The dissolution and regeneration of bagasse in the recycled [C_4mim]Cl were performed under the same conditions, and the results are listed in Table 1. Compared with that in the fresh [C_4mim]Cl, the dissolution of bagasse in the recycled [C_4mim]Cl was still achieved within 4 h; however, the yield of regenerated bagasse (residue 1) decreased. The similar decreased pretreatment efficiency of lignocelluloses was reported in the recycled [Amim]Cl.[23] ^{1}H and ^{31}P NMR analyses of the fresh and recycled [C_4mim]Cl were performed to investigate the possible reason for the decreased residue 1. Figure 3 illustrates the ^{1}H

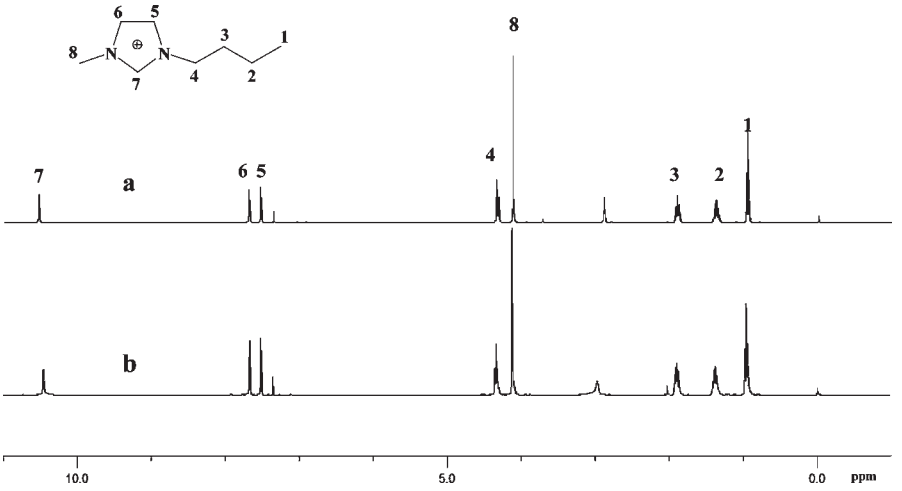

Figure 3. ^{1}H NMR spectra of the fresh (spectrum a) and recovered [C_4mim]Cl (spectrum b, third cycle).

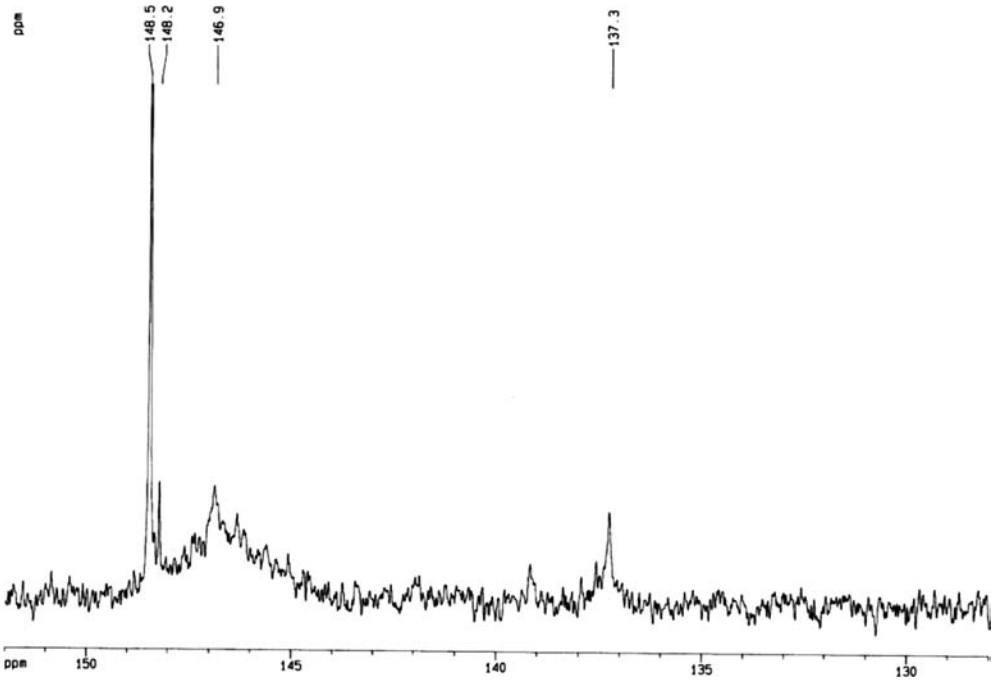

Figure 4. ^{31}P NMR spectrum of the recycled [C_4mim]Cl with third reuse cycle.

NMR spectra of the fresh and recycled [C_4mim]Cl with the third reuse cycle. The results from ^{1}H NMR analysis showed that there was no obvious difference between fresh and recycled [C_4mim]Cl, which indicated that the recycled IL contained only trace amounts of contaminants that could not be detected with ^{1}H NMR analysis. According to a previous paper,[23] these contaminants were probably derived from the degradation of [C_4mim]Cl and lignocelluloses. In addition, they were also probably introduced with chemicals, water, and other solvents. Figure 4 shows the ^{31}P NMR spectrum of the recycled IL with the third recycle. Compared with the fresh IL, two small signals at 137.3 and 146.9 ppm were observed. The former signal originated from −COOH, and the latter from aliphatic −OH. These contaminants were liberated from the degradation of lignocelluloses, especially from hemicelluloses and cellulose. These two weak signals could not be collected in the first recycle [C_4mim]Cl, indicating the trace amount in each recycle and accumulation with the increased recycle number. In addition, the total lignin content of the regenerated bagasse increased with the improved recycle number, which was probably due to the degradation of carbohydrate catalyzed with the enriched organic acid contaminants in the recycled IL. The similar decreased hydrolysis or separation efficiency in the recycled ILs was previously reported because of the presence of organic acid and other degraded substances.[23,26−28] These results indicated that more effective methods for ionic liquid recycling should be further investigated with increased recycle numbers.

Lignin Content of Polysaccharide-Rich Fractions. The acid-soluble and acid-insoluble lignin contents of the original bagasse and the polysaccharide-rich fractions including residues 1, 3, and 4

Table 2. Lignin Contents of the Original Bagasse and Polysaccharide-Rich Fractions from Bagasse

fraction	lignin content[a] (%)		
	L_{AI}[b]	L_{AS}[c]	L_T[d]
bagasse	17.02	2.22	19.24
residue 1 (carbohydrate-rich material)	10.09	0.53	10.62
residue 3 (cellulose)	4.01	0.55	4.56
residue 4 (hemicellulose)	2.55	0.99	3.54

[a] Based on the dried fractions. [b] L_{AI}, acid-insoluble lignin. [c] L_{AS}, acid-soluble lignin. [d] L_T, total lignin.

Table 3. Contents of Neutral Sugars and Uronic Acids in the Cellulosic (Residue 3) and Hemicellulosic Fractions (Residue 4)

sugar	residue 3	residue 4
rhamnose	tr[a]	0.08
glucose	92.15	16.58
xylose	6.23	73.25
mannose	0.51	tr
arabinose	0.43	8.31
galactose	0.09	0.80
glucuronic acid	0.20	0.72
galacturonic acid	0.39	0.26

[a] tr, trace amount.

were determined according to NREL 2008 standard, and the results are listed in Table 2.

Compared with the original bagasse, residue 1 regenerated in acetone/water after dissolution in [C_4mim]Cl had a much lower lignin content (10.62%) with a reduction of 44.08% of the original lignin, which was probably due to the removal of lignin without linking to carbohydrates, indicating that dissolution in [C_4mim]Cl followed by precipitation in acetone/water achieved fairly good delignification. Similar results were reported by Lee et al.[27] They extracted about 44% of lignin from wood flour with [C_2mim]Ac at 110 °C. Fu et al.[29] isolated much more lignin (52.7% of acid-insoluble lignin) from triticale straw with [C_2mim]Ac at higher temperature (150 °C). Alkaline extraction of residue 1 with 3% NaOH led to further decreases in lignin content to 4.56 and 3.54% for residues 3 and 4, respectively, suggesting some ether linkages between carbohydrates and lignin could not be cleaved under the alkaline condition used.

Sugar Composition and Content of Polysaccharide Fractions. The neutral sugar composition and uronic acid content of the polysaccharide fractions (residues 3 and 4) are given in Table 3. Clearly, glucose (92.15%) was the predominant sugar in cellulosic residue 3, indicating the high purity of cellulose. The cellulose also contained a noticeable amount of noncellulose sugars (6.23% xylose). Minor amounts of mannose (0.51%), arabinose (0.43%), and galactose (0.09%) and a trace amount of rhamnose were also observed in cellulose. Uronic acids, including glucuronic acid (0.20%) and galacturonic acid (0.39%), also appeared in minor quantities. These data confirmed that although most of the available hemicelluloses were extracted with 3% NaOH, some parts of hemicellulosic polymers were still strongly resistant to alkaline extraction under the conditions used, implying the hemicelluloses present not only in the surface of cellulose but also in the pores of fibrils network can be retained during alkaline extraction. The relatively high purity of the cellulosic fraction (over 92%) was due to the easy extraction of the noncellulose components with this fractionation method.

In hemicellulosic residue 4, xylose (73.25%) was the major sugar component. Glucose (16.58%) and arabinose (8.31%) were observed in noticeable amounts. Rhamnose, galactose, and uronic acid were identified in minor quantities (0.08−0.80%), and only a trace amount of mannose was detected. These results indicated that the isolated hemicellulosic fractions contained cellulose degraded during dissolution in IL and extraction with alkali.

Table 4. Weight-Average (M_w) and Number-Average (M_n) Molecular Weights and Polydispersity (M_w/M_n) of MWL and the Isolated Lignin Fractions from Bagasse

	MWL	acetone-soluble lignin (residue 2)	alkaline lignin (residue 5)
M_w	13720	9090	7817
M_n	4101	2902	1886
M_w/M_n	3.345	3.133	4.145

Molecular Weight of Lignin. MWL has been considered as the standard preparation representing the original lignin. In the present study, MWL was prepared from bagasse, and the isolated lignin fractions were comparatively studied with MWL. The weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of MWL, residue 2 (acetone soluble lignin), and residue 5 (alkaline lignin) are listed in Table 4. The M_w of bagasse MWL was 13720. The M_w of acetone soluble lignin (residue 2), obtained after dissolution in [C_4mim]Cl at 110 °C followed by precipitation in acetone/water (9:1, v/v), decreased to 9090. It was probably due to the partial cleavage of β-O-4 linkages between the lignin precursors under the condition used. On the other hand, it was reported that MWL had a high proportion of bonded hemicelluloses,[30] which resulted in the increased molecular weight of MWL. However, in the present study, most of the lignin isolated with acetone/water from bagasse/[C_4mim]Cl homogeneous solution was that without linking to carbohydrates. These free lignin macromolecules had lower molecular weights than those with carbohydrates attached. In addition, the M_w/M_n of residue 2 (3.133) was slightly smaller than that of MWL (3.345) because of the absence of lignin with carbohydrates attached. The M_w of alkaline lignin, residue 5, further decreased to 7817. This decrease was probably due to the cleavage of interunit bonds at the alkaline conditions used. The M_w/M_n of residue 5 (4.145) also increased compared with that of MWL because of the degradation. These results were consistent with the relatively high content of glucose in the isolated hemicellulosic fraction.

FT-IR Spectra. In the present study, the prepared MWL and the commercially available MCC and xylan were selected as the standard lignin, cellulose, and hemicelluloses, respectively. The physicochemical properties of the isolated cellulose, hemicelluloses, and lignin from bagasse were comparatively studied with these standard materials. Figure 5 illustrates the FT-IR spectra of MCC (spectrum a) and cellulosic residue 3 (spectrum b). In the two spectra, the absorbances at 3441−3344, 2895, 1636, 1429−1420, 1375, 1313, 1162, 1112, 1057, and 893 cm^{-1} are all associated with native cellulose. The absorption at 3441−3344 cm^{-1} is due to the O−H stretching and that at 2895 cm^{-1} to the C−H stretching. The band at 1636 cm^{-1} is associated with

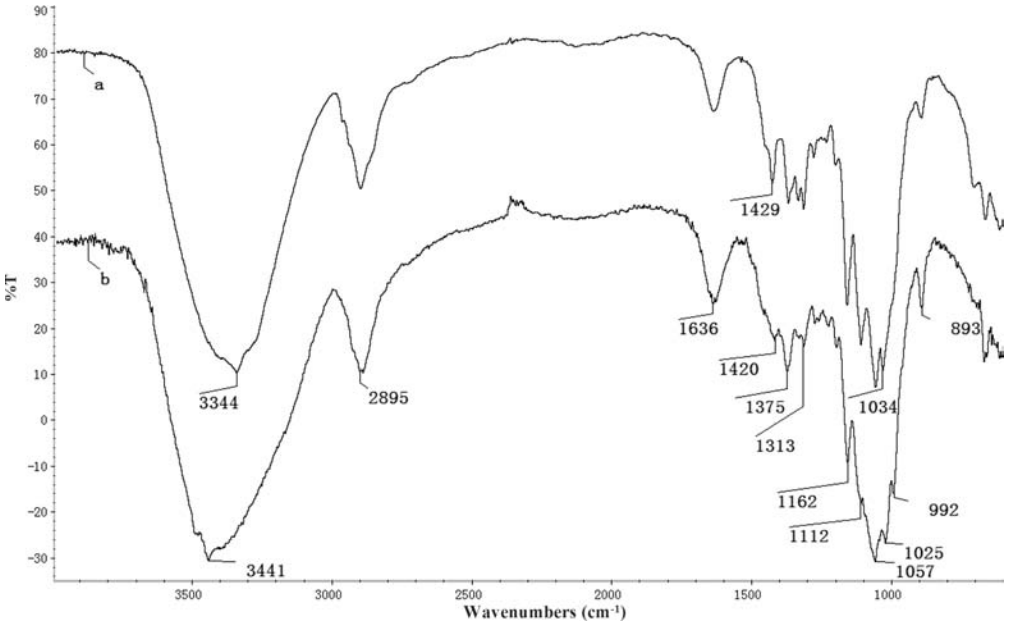

Figure 5. FT-IR spectra of MCC (spectrum a) and the isolated cellulosic fraction from bagasse (residue 3, spectrum b).

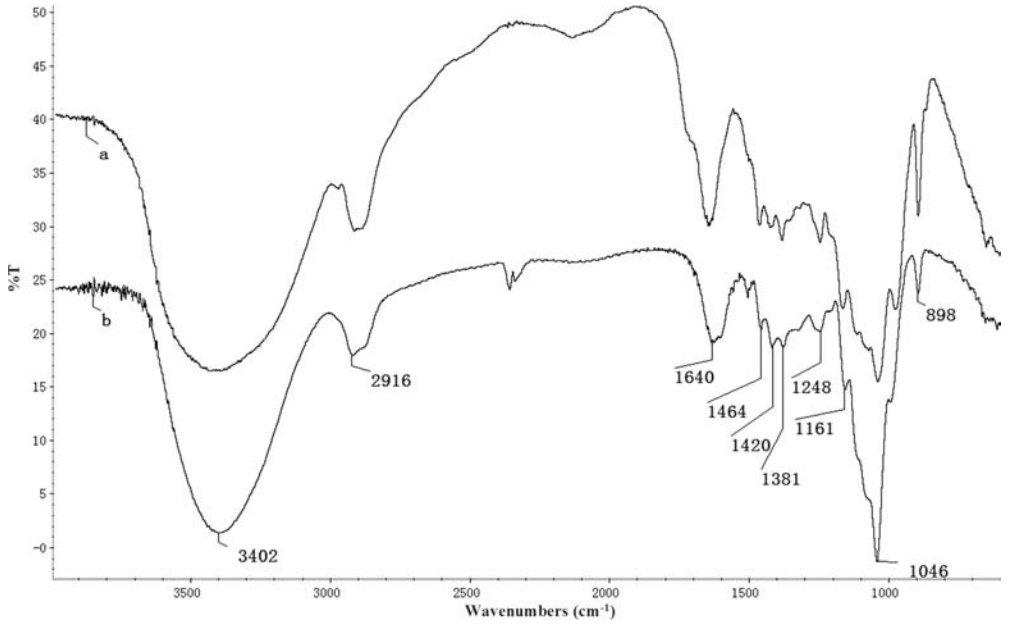

Figure 6. FT-IR spectra of xylan from oat spelts (spectrum a) and the isolated hemicellulosic fraction from bagasse (residue 4, spectrum b).

the bending mode of absorbed water. A small peak at 1429 cm^{-1} relates to the CH_2 symmetric bending. The absorbances at 1375 and 1313 cm^{-1} originate from the O−H bending and C−H bending. The peak at 1162 cm^{-1} is attributed to C−O antisymmetric stretching. A shoulder band at 1112 cm^{-1} relates to C−OH skeletal vibration. The C−O−C pyranose ring skeletal vibration gives a prominent band at 1057 cm^{-1}. A small sharp peak at 893 cm^{-1} corresponds to the glycosidic C_1−H deformation with ring vibration contribution, which is characteristic of β-glycosidic linkages between glucose in cellulose. The similar pattern of these two spectra indicated the similarity in chemical structure between MCC and cellulosic residue 3.

The FT-IR spectra of xylan (spectrum a) and hemicellulosic residue 4 (spectrum b) are illustrated in Figure 6. The peaks at 3402, 2916, 1640, 1464, 1420, 1381, 1248, 1161, 1046, 978, and 898 cm^{-1} are attributed to hemicelluloses, in which 1161 and 1046 cm^{-1} are typical of arabinoxylans.[31] The presence of the arabinosyl side chains is documented by two weak shoulders at 1161 and 978 cm^{-1}. The changes of intensity for these two bands suggested an arabinosyl substituent contribution. The lignin-related band at 1509 cm^{-1} was observed in spectrum b, which was consistent with the results in Table 2. The absence of an adsorption shoulder at 1710 cm^{-1} for a carbonyl group in spectrum b implied that the hemicellulosic fraction extracted with 3% NaOH was free of acetyl groups.

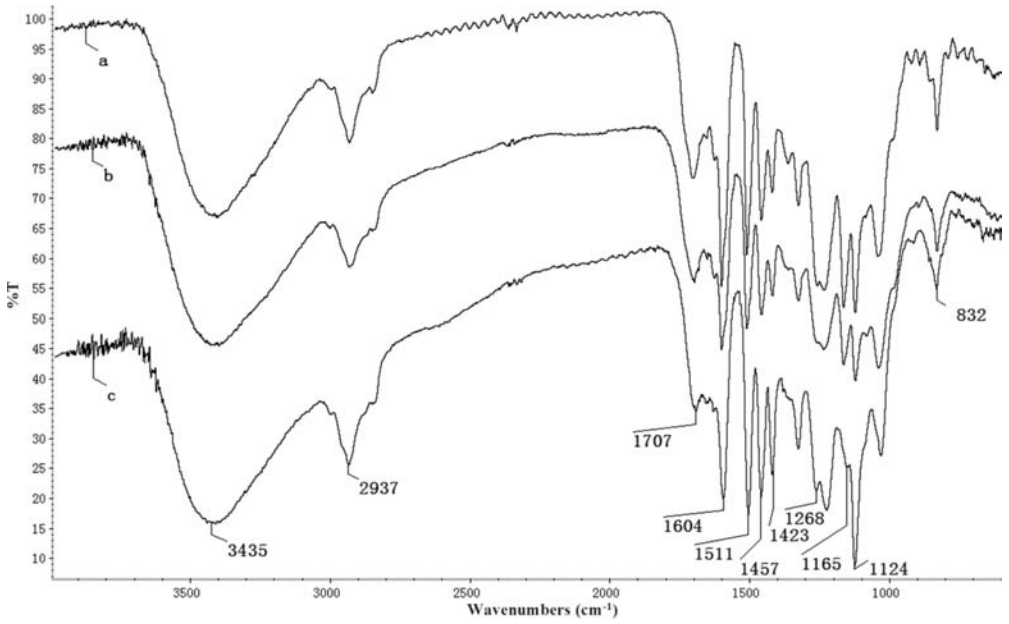

Figure 7. FT-IR spectra of MWL (spectrum a), acetone-soluble lignin fraction (residue 2, spectrum b), and alkaline lignin (residue 5, spectrum c).

The FT-IR spectra of MWL (spectrum a) and lignin fractions isolated with acetone/water (residue 2, spectrum b) and 3% NaOH (residue 5, spectrum c) are illustrated in Figure 7. The absorption band at 1707 cm^{-1} for C═O stretching indicates the presence of hydroxycinnamates, such as *p*-coumarate (PCA) and ferulate (FA).[32] Compared with spectrum a, the intensity of this band decreased in spectra b and c, indicating the decreased content of hydroxycinnamates in the isolated lignin fractions. Ferulate esterified with an arabinosyl unit in hemicelluloses was attached to lignin with an ether bond to form a carbohydrates−ferulate−lignin bridge structure.[33] During precipitation of bagasse/[C_4mim]Cl solution in acetone/water, lignin containing this bridge structure could not be dissolved in acetone/water, resulting in the decreased content of FA in acetone-soluble lignin fraction (residue 2). PCA is always esterified with lignin and hemicelluloses in cell walls, especially in nonwood materials including bagasse. The formed ester could be saponified under alkaline conditions, leading to the decreased content of PCA in the alkaline lignin fraction (residue 5) compared with MWL. On the other hand, the similar intensities of the bands at 1604, 1511, and 1423 cm^{-1} for aromatic skeletal vibrations and that at 1457 cm^{-1} for C−H deformations and aromatic indicated the similar core structures of MWL and the lignin fractions solubilized in acetone/water (9:1, v/v) and 3% NaOH, implying that these two lignin fractions had no obviously different core aromatic structure except the linkages to carbohydrates. In addition, the band at 1165 cm^{-1} for the C−O antisymmetric bridge stretching in ester significantly decreased in spectrum c, which was probably due to the noticeable saponification of esters between lignin and hydroxycinnamates during alkaline extraction.[32]

X-ray Diffraction Spectra. A diffractogram of native bagasse, ball-milled bagasse, and separated cellulose (residue 5) is illustrated in Figure 8. The native bagasse is of cellulose I, indicated by the presence of the typical diffraction peaks of the (200), (110), (1$\bar{1}$0) and (004) planes. The stong peak at 22.12° corresponds to the (200) plane of crystals, the diffraction peaks of the (110) and (1$\bar{1}$0) planes merged together at 16.13°, and the diffraction peak (004) at 34.42° is very weak. After ball-milling for 72 h, the intensity of the (200) peak dramatically decreased, and the peaks for the (110), (1$\bar{1}$0), and (004) planes disappeared. Only a broad diffused diffraction peak around 21.16° is observed. Obviously, the crystalline structure of cellulose in native bagasse was significantly destroyed by ball-milling for 72 h. However, the cellulosic residue 5 isolated from ball-milled bagasse by dissolution and alkaline extraction has the crystal form of cellulose II according to the peaks at 12.14° for (1$\bar{1}$0), 20.16° for (110), and 21.80° for (200). This indicated that cellulose macromolecules were recrystallized as cellulose II after the removal of amorphous hemicelluloses and lignin in ionic liquid solution followed by alkaline extraction.

^{1}H NMR Spectra. Nuclear magnetic resonance has significant advantages for understanding complicated structure. To obtain further information of the hemicellulosic fraction extracted with 3% NaOH solution from bagasse, it was analyzed by ^{1}H NMR spectrometry in D_2O, and the spectrum is illustrated in Figure 9. Obviously, a strong signal at 4.7 ppm originated from the residual solvent (HDO). The anomeric protons give signals at 4.3−4.5 ppm for β-configuration and at 4.9−5.4 ppm for α-configuration.[31] The peak at 5.34 ppm originated from the anomeric protons of terminal α-L-arabinofuranosyl residue linked to O-2 and O-3 of the xylopyranosyl residues,[34,35] and that at 5.23 ppm is due to the anomeric protons of 4-*O*-methyl-D-glucuronic acid. The signals at 4.44/4.42 ppm relate to the anomeric protons of β-(1→4)-D-xylose. The peaks at 4.23/4.21, 4.11, and 3.86 ppm are associated with H_2−H_5 in the α-L-arabinofuranosyl unit.[36] The small signal at 1.2 ppm (not shown) relates to the CH_3 of a small amount of 4-*O*-methyl-D-glucuronic acid. The weak peak at 6.4 ppm (not shown) originates from lignin, corresponding to the presence of lignin in Table 2. Previous studies showed that the water-soluble hemicelluloses were more branched and rich in glucose, whereas the alkali-soluble hemicelluloses consisted mainly of glucuronoarabinoxylans or L-arabino-(4-*O*-methylglucurono)-D-xylans, especially for those extracted with higher concentrations of

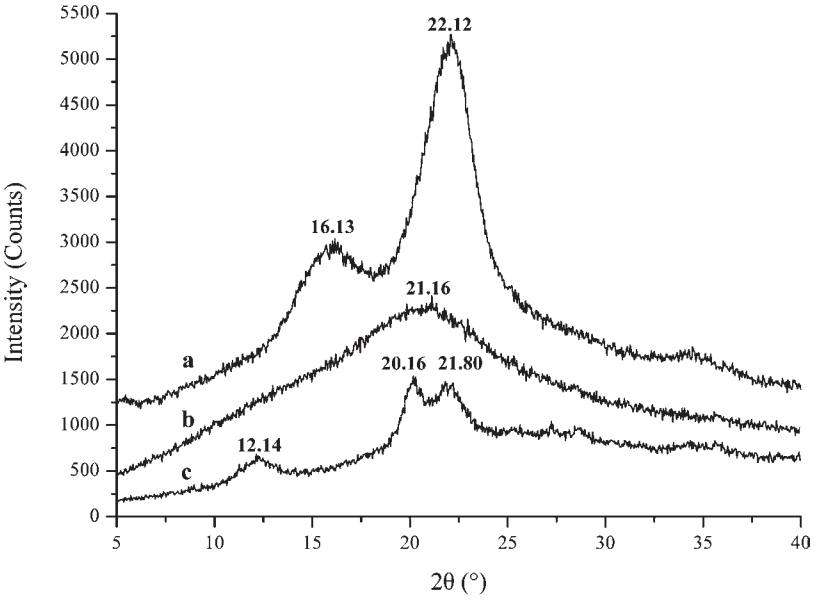

Figure 8. X-ray diffraction patterns of native bagasse (a), ball-milled bagasse (b), and isolated cellulosic residue 5 (c).

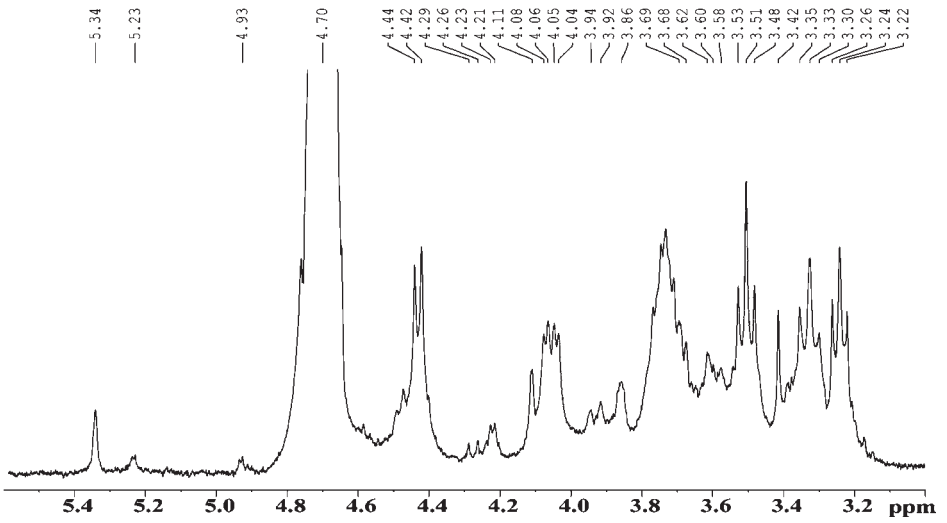

Figure 9. ^{1}H NMR spectrum of hemicellulosic fraction from bagasse (residue 4).

NaOH solution.[31] According to sugar composition and FT-IR and ^{1}H NMR analyses, hemicellulosic residue 4 extracted from bagasse with 3% NaOH solution after dissolution in [C_4mim]Cl and precipitation in acetone/water contained mainly 4-*O*-methyl-D-glucuronoxylans with some α-L-arabinofuranosyl residue substituted at C-2 and C-3.

HSQC NMR Spectra. To better understand the detailed structures of the isolated lignin fractions, the acetylated MWL and the lignin fractions (residues 2 and 5) were comparatively analyzed using 2D ^{1}H−^{13}C HSQC NMR techniques. The spectra and the corresponding predominant substructures are clearly differentiated in Figure 10. Therefore, the side chain or aliphatic region (upper right of each panel) and the aromatic region (lower left of each panel) of the NMR spectra provide clear structural information.

In the aliphatic region, signals of typical lignin units such as β-O-4 (A, red color) and β-5 (B, orange color) can easily be seen. Clearly, the β-O-4 ether units are the major interunit structures. Methoxyl groups and cinnamyl alcohol end-groups were also observed in this region. In addition, some of the signals belonging to carbohydrate components were detected, indicating that small amounts of carbohydrates were present in the lignin fractions. Obviously, the sugar content in the isolated lignin fractions was lower than that in MWL. In the aromatic region, syringyl (S) and guaiacyl (G) units with traces of *p*-hydroxyphenyl (H) units were observed in the spectra of residues 2 and 5. The signals from H were also detectable at lower contour levels (not shown) in the spectrum of MWL. In addition, strong signals originating from *p*-coumaric acid were detected,[37] indicating the presence of a large amount of PCA in bagasse. The similar HSQC spectra of MWL and the isolated lignin fractions confirmed that the structures of the two lignin fractions were similar. This result was consistent with the results obtained from FT-IR.

In conclusion, the complete dissolution of bagasse in [C_4mim]Cl and the precipitation of the resulting solution in acetone/water (9:1, v/v) followed by extraction of the regenerated carbohydrate-rich material with 3% NaOH resulted in the

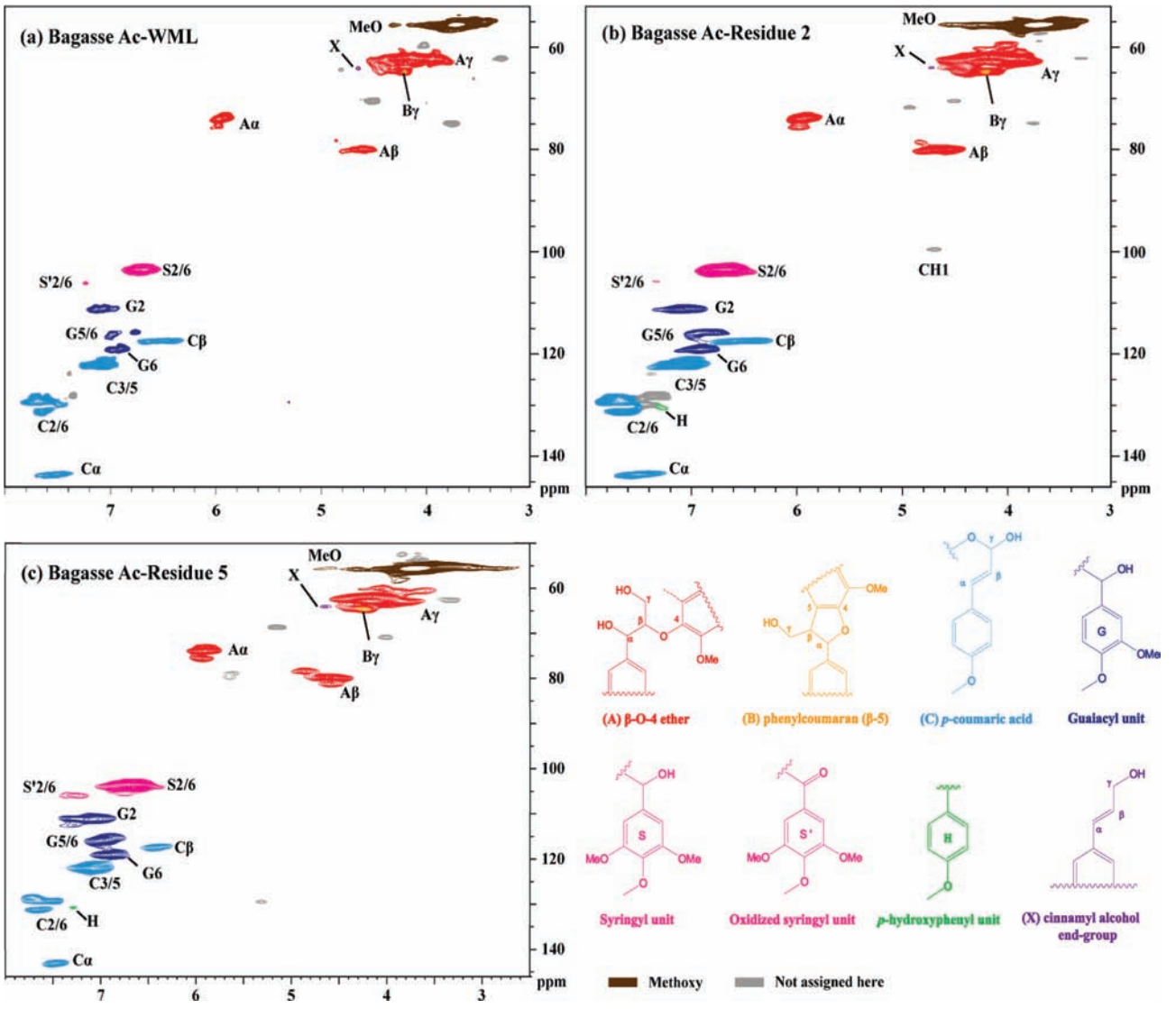

Figure 10. HSQC spectra of MWL (spectrum a), acetone-soluble lignin (spectrum b), and alkaline lignin (spectrum c) as well as lignin primary substructures.

fractionation of bagasse to 36.78% cellulose, 26.04% hemicelluloses, and 10.51% lignin, accounting for 47.17 and 33.85% of the original polysaccharides and 54.62% of the original lignin, respectively. Chemical analysis and FT-IR and NMR analyses indicated that the structures of the isolated lignin fractions were similar to those of MWL. The easy extraction of the noncellulose components with this fractionation method resulted in the relatively high purity of cellulosic fractions (over 92%). The hemicellulosic fraction was mainly 4-*O*-methyl-D-glucuronoxylans with some α-L-arabinofuranosyl residue linked to main chain at C-2 and C-3. More efforts are still required to optimize the fractionation conditions to increase the yields and purity of the isolated components. In addition, more effective methods for ionic liquid recycling should be further investigated with the increased recycle numbers.

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